PHYSIOLOGY OF SULFATE IN HIGHER PLANTS III. SULFATE UPTAKE AND METABOLISM IN BARLEY AS INFLUENCED BY LIGHT AND DARK

PHYSIOLOGY OF SULFATE IN HIGHER PLANTS III. SULFATE UPTAKE AND METABOLISM IN BARLEY AS INFLUENCED BY LIGHT AND DARK

By

Kenji TORII and Akio FUJIWARA

Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University, Sendai, Japan

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The most characteristic symptoms of sulfur deficient plant are chlorotic appearances of the leaves. Many analytical results indicate that the chlorophyll concentration is lower in chlorotic than in normal leaves. Since chlorophyll contains no sulfur, Smith (1) indicated that chlorosis due to lack of sulfur must be an indirect effect of deficiency unless the sulfur containing chloroplast proteins are directly involved in the synthesis or stability of chlorophyll which is attached to the protein as a prosthetic group.

Absorption, translocation and assimilation of nutrients, generally, require the expenditure of energy. The only energy source in plants is photosynthesis. The determining stage of the root nutritive process is not only the absorption, but the assimilation of mineral nutrients, i.e., in which their inclusion in the composition of organic compound and involvement in metabolism of organic substances have been recognized. However, the primary source of the above process is also photosynthesis.

These general aspects are confirmed by the experimental data of many investigators (2–4), who showed a direct relationship between the assimilation rate of mineral nutrients and the presence or absence of light (and, consequently, also of photosynthesis) either under natural or by artificially substituted conditions.

This was shown clearly in relation to nitrate assimilation and sulfate assimilation (5, 6). A number of the investigators relate the better nitrate or sulfate assimilation in light, to the fact that, apart from their customary reduction in darkness, there exists also a specific photochemical reduction, closely related to the activity of the photosynthetic mechanisms.

The mechanism of the yellowing of a green plant in dark condition is different from the mechanism of chlorosis formation in the environmental change of a plant.

* Present address: Government Forest Experiment Station, Meguro, Tokyo, Japan

However, it will be important to investigate how sulfur metabolism reacts under conditions of light and darkness in order to understand the theories that evolve in the relationship between photosynthesis and sulfur nutrition.

Experiment 1. Effect of shading on the utilization of sulfur in green plant of barley.

Barley seeds were germinated and cultured in normal condition as previously described (7) during two weeks. The seedlings were in the stage of developing the second leaf. Radioactive sulfate ($Na_2{}^{35}SO_4$) was introduced to the plants through their roots in sulfur deficient nutrient solution. The nutrient solution contained radio-sulfur as 2000 cpm/ml of solution with carrier sulfate as 25 ppm sulfur. Culture of plant was conducted in a culture chamber at 27°C with artificial fluorescent light (4000 lux) in the case of light condition and without light for the dark condition. After appropriate periods, seedlings were taken out from nutrient solution and the free space sulfate of roots was washed out with distilled water, and then the shoot and root were separately subjected for analysis of radioactivity in the different subcellular components. The methods of the analysis of radioactivity and fractionation of the subcellular components were the same as previous experiment (7). After homogenizing the tissue of the plant with trichloroacetic acid solution, the radio-sulfur of tissue was fractionated into; crude protein-^{35}S, soluble organic-^{35}S and inorganic sulfate-^{35}S.

Results

An initial experiment was conducted to determine the rate of uptake of ^{35}S-sulfate into seedlings. The first column of Table 1 showed the total ^{35}S activity of plants during 48 hours periods. The uptake of radio-sulfate was not affected with different light treatment. The dark treatment of the plant did not change the rate of sulfate uptake and just a slight increase was found in a later period of the experiment. However, in the second column of Table 1, it was shown that the transport of the absorbed sulfate from root to shoot was disturbed by the dark treatment. The degree of the disturbance of transport into the shoot was higher under the dark condition. The transport of radio-sulfate into the shoot was decreased by the lack of light supply.

The behavior of radio-sulfate uptake under light and dark conditions was studied further by means of crude fractionation of the subcellular components of the plant tissues. In the results of the shoot, it can be seen that transported radio-sulfur into the shoot, while the rate of transport was disturbed by the dark condition, are accumulated relatively more into crude protein fraction at dark than at light during early periods of the experiment (1 to 5 hours). However, at later periods (5 to 48 hours), the accumulation into crude protein fraction became higher under light conditions and lower in dark. From the results of radio-

Table 1. Effect of darkness (complete shading) on uptake of ^{35}S-sulfate by green barley seedlings.

Total ^{35}S absorbed (cpm/10 plants)							
Light (control)		370	540	1170	2060	7450	10870
Dark		490	540	1130	2110	7650	13300
Ratio of ^{35}S absorbed into shoot and root (%)							
Light	Shoot	6.7	9.7	24.6	27.2	26.1	27.2
	Root	93.3	90.3	76.0	72.6	73.9	72.8
Dark	Shoot	1.8	3.8	16.0	20.7	26.9	22.8
	Root	98.2	96.2	84.0	79.3	73.1	77.2
^{35}S distribution in the cellular components of shoot (%)							
Light	Protein-^{35}S	8.0	7.7	6.0	9.5	12.5	12.3
	Amino-^{35}S	—	—	—	0.9	0.6	1.2
	SO_4-^{35}S	92.0	92.3	94.0	90.1	86.8	86.5
Dark	Protein-^{35}S	7.7	9.7	9.5	8.5	8.9	10.6
	Amino-^{35}S	—	—	0.9	0.4	0.6	4.2
	SO_4-^{35}S	92.3	90.3	90.1	90.1	90.5	88.2
^{35}S distribution in the cellular components of root (%)							
Light	Protein-^{35}S	14.4	13.2	14.8	13.0	11.2	11.6
	Amino-^{35}S	5.0	7.8	8.0	6.4	5.3	5.2
	SO_4-^{35}S	80.6	79.0	77.2	80.6	83.5	83.2
Dark	Protein-^{35}S	15.6	13.6	14.8	13.9	7.6	8.2
	Amino-^{35}S	6.2	8.0	4.8	5.3	5.2	4.8
	SO_4-^{35}S	78.2	78.4	80.4	80.8	87.2	87.0

sulfur assimilation into cellular components of root tissue, about the same tendencies of radio-sulfur assimilation like that in the shoot can be observed. In other words relatively higher assimilation into crude protein fraction and into soluble organic-^{35}S fraction at early periods of the experiment by means of dark treatment, and higher assimilation of inorganic sulfate-^{35}S by means of the prolonged periods of dark treatment were revealed.

Experiment 2. Effect of light on the uptake and utilization of ^{35}S-sulfate by etiolated barley seedlings.

Etiolated barley seeldings were prepared in a culture chamber at 30°. Barley seeds (Shoki variety) were germinated in distilled water and then cultured in nutrient solution as previously described (7) for 10 days at complete darkness. At this period, the seedlings developed the second leaf and revealed a complete etiolation with yellowish leaves. The introduction of the radioactive^{35}S-sulfate was

conducted by means of adding ^{35}S-Na_2SO_4 to the nutrient solution (4000 cpm/ml of solution with 5 ppm carrier sulfate). After appropriate periods, sample seedlings were separated into shoots and roots and then each of the tissues was fractionated into three subcellular components: 1; particulate and cell wall fraction, 2; cytoplasmic soluble protein fraction, 3; soluble non-protein fraction, by means of the methods of centrifugal fractionation as described before (8).

Results

Table 2 showed that the sulfate uptake was affected by supplying light during the re-greening process of etiolated barley seedlings. Compared with the etiolated seedling which was kept under dark, re-greening barley seedling take up less sulfate during 48 hours experiment periods. The second column of Table 2 shows that the transport of sulfur from root to shoot was more intensive in the re-greening plant than in the dark plant. It was shown in Table 2 that the transported radio-sulfur in the shoot was assimilated more into the particulate fraction and into the cytoplasmic protein fraction in the re-greening plant, but in the dark plant the transported sulfur to the shoot was assimilated in soluble non-protein fraction.

Experiment 3. Effect of shading on the re-utilization of the absorbed ^{35}S-sulfate in green barley seedlings after transferring them to a non-radioactive nutrient solution.

In preceding experiments, ^{35}S-sulfate was supplied continuously throughout the experimental periods. It is difficult to investigate the direct relationships between root sulfur and shoot sulfur, because we could not recognize the difference between ^{35}S-sulfate which once assimilated into subcellular components of root and ^{35}S-sulfate directly came through the root without being metabolically involved. In this experiment, the authors want to re-examine this point by means of transferring ^{35}S-sulfate treated plants to non-radioactive nutrient solution and cultured further to understand the effect of shading on the re-utilization of absorbed ^{35}S-sulfur in green barley seedlings.

Green barley seedlings were prepared as in the Experiment 1. Radiaoctive sulfate (^{35}S–Na_2SO_4; 4000 cpm/ml of the solution) was introduced to the plants for 24 hours under continuous light conditions and then the plant was transferred to the non-radioactive normal nutrient solution under light or dark conditions. The plants were taken out from the solution at each appropriate interval and an analysis of the radioactivity was followed as previously described. Fractionation of the subcellular components was carried out to separate the tissues into four fractions; cell wall and cell debris fraction (F-a), particulate fraction (F-b), cytoplasmic soluble protein fraction (F-c) and cytoplasmic non-protein fraction (F-d). The method of the fractionation was the same as the previous experiment (8).

Table 2. Effect of light supply on the uptake of ^{35}S-sulfate by etiolated barley seedlings.

Absorption periods		2 hrs.	10 hrs.	48 hrs.
Light		970	1400	10250
Dark (control)		1015	3080	14100
Ratio of ^{35}S absorbed in shoot and root (%)				
Light	Shoot	37.9	45.2	51.2
	Root	62.1	54.8	48.8
Dark	Shoot	38.0	32.6	40.3
	Root	62.0	67.4	59.7
^{35}S distributed in the cellular components of shoot (%)				
Light	Particulates	16.7	26.8	40.5
	Cytoplasmic	5.8	9.6	12.0
	Soluble	73.5	63.6	47.5
Dark	Particulates	8.0	10.1	18.4
	Cytolasmic	2.0	3.1	3.4
	Soluble	90.0	86.8	78.2

Results

Experiments were arranged for the time course experiment for 72 hours duration. However, in Table 3, only the results of the experiment in which the plant cultured in a non-radioactive nutrient solution for 72 hours are presented. From Table 3, it can be seen that the total radioactivity in the plant was decreased by the shading of the plant, presumably the high rate of exudation of absorbed ^{35}S-sulfur into the solution by means of the shading were recognized. The transport of radio-sulfur from root to shoot was disturbed by the shading as previously discussed in the preceding section. In this experiment, the leaves of the plant were separated into young and old leaves. It is interesting that, with a lack of light supply, the transport of radio-sulfur was dominant into the young leaves and, while under a continuous light condition, dominant in old leaves. In Table 4, the distribution patterns of absorbed sulfur into the cellular components of leaves are explained. During 72 hours incubation, absorbed ^{35}S-sulfur was accumulated into the cellular components. By shading treatment, however, transported ^{35}S-sulfur in young leaves was not utilized for chloroplast fraction (F-b) and cytoplasmic soluble protein fraction (F-c). Under these conditions, sulfur was accumulated in cytoplasmic non-protein fraction (F-d). On the contrary, under a continuous light condition, young green leaves were transported less amount of ^{35}S-sulfur from the root, but the accumulation of sulfur into the cellular components, e.g. (F-b) and (F-c), was

Table 3. Effect of the darkness (complete shade) on the translocation and re-utilization of absorbed ^{35}S-sulfur in green barley seedlings. (After 72 hrs. supply of $^{35}SO_4$)

	Total ^{35}S	cpm in young leaf	cpm in old leaf	Ratio of $\frac{\text{shoot}}{\text{root}}$	Exudate in outer soln.
0 time sample*	29951**	7995 (42.9)	10605 (57.1)	$\frac{62.2}{37.8}$	0
Light	22330	6490 (33.8)	10120 (66.2)	$\frac{78.0}{22.0}$	7610
Dark	20250	8190 (53.6)	7022 (46.4)	$\frac{72.0}{28.0}$	9300

* 0 time sample means the sample before giving dark treatment
** (cpm/15 seedlings)

Table 4. Effect of the darkness on the intracellular distribution of absorbed ^{35}S-sulfur after 72 hrs. in non-radioactive nutrient solution.

	Light		Dark		O-time sample	
	Young	Old	Young	Old	Young	Old
(F-a)	8.2*	5.2	8.2	10.1	3.2	4.0
(F-b)	35.9	25.3	20.0	40.6	11.8	34.0
(F-c)	27.8	13.0	28.2	24.2	12.2	32.4
(F-d)	28.1	56.4	43.6	21.1	72.8	30.6

* % rate to total.

evidently higher. Moreover, in old leaves, the lack of light supply made for the increasing of the accumulation of sulfur into (F-b) as compared with the continuous light condition.

Experiment 4. Effect of light on the re-distribution of absorbed ^{35}S-sulfur in the excised green or yellow (etiolated) barley leaves.

In this experiment, to avoid the effects of ^{35}S-sulfur in root on the sulfur metabolism of the shoot, the leaves of barley seedlings were excised from the root after the introduction of the ^{35}S-sulfate through the root and kept for 12 hours on a moistened paper in a Petri dish. After the seeds were germinated in the dark, the barley seedlings were cultured in distilled water at 27°C for 5 days with artificial light in the case of green plant culture and without light in the case of etiolated seedling preparation. Both green and etiolated seedlings were supplied radioactive ^{35}S-sulfate ($Na_2{}^{35}SO_4$, 2000 cpm/ml of nutrient solution with 3 ppm SO_4 as carrier) in sulfur deficient nutrient solution of previous experiment (7) for a 30 hour duration. After the above treatments, the leaves of both green and etiolated seedlings were cut off and incubated in a Petri dish which contained a moistened filter paper. The incubation was conducted at 30°C with and without light. The methods of the fractionation of cellular components were the same as in the preceding section.

Results

Table 5 and Table 6 show the results of the experiments with excised leaves. The results from the cellular ^{35}S-sulfur analysis of green excised leaves which were treated during 12 hours under the light and dark in non-radioactive nutrient solution are shown in Table 5. During 12 hour incubation periods, the dry weight of (F-b) and (F-c) was slightly increased in the plant tissue with continuous light treatment, but in the plant tissue without light supply, the dry weight of (F-b) and (F-c) was decreased while increasing of dry weight of (F-d). Similar tendencies can be recognized in the results of ^{35}S-sulfur distribution patterns. By means of shading, the utilization of ^{35}S-sulfur into the cellular fractions of green leaves were inhibited in (F-b) and (F-c). Only the green plant leaves with a continuous supply of light, sulfur assimilation to the cellular components (particulate matters or cytoplasmic soluble protein fraction) from soluble non-protein fraction (F-d) was continued. In Table 6, the results of the effect of light supply on the etiolated leaves involving sulfur utilization are presented. It can be seen that the etiolated leaves synthesized (F-b) and (F-c) utilizing the soluble non-protein sulfur fraction during the greening process while the etiolated leaves did not show any particular increase of the dry weight or the incorporation of ^{35}S-sulfur into (F-b) or (F-c). This may mean that the assimilation of sulfur and synthesis of the chloroplasts have a direct relation in their metabolic process. The details of this consideration will be discussed later.

Table 5. Effect of the darkness on the re-distribution of absorbed ^{35}S-sulfur in the excised green barley leaves. (30°C. 12 hrs. incubation)

Fraction	O-time sample		Light		Dark	
	Dry wt.	^{35}S-rate	Dry wt.	^{35}S-rate	Dry wt.	^{35}S-rate
(F-a)	83.4*	2.8**	109.0*	3.4**	99.2*	5.1**
(F-b)	49.3	15.0	50.1	16.8	47.3	18.4
(F-c)	33.8	18.9	32.1	40.8	29.3	17.8
(F-d)	104.6	63.3	117.8	39.4	115.2	58.7

* Dry weight. mg/33 plants; **% rate of total.

Table 6. Effect of light supply on the re-distribution of absorbed ^{35}S-sulfur in the excised etiolated barley leaves. (30°C. 12 hrs. incubation)

Fraction	O-time sample		Light		Dark	
	Dry wt.	^{35}S-rate	Dry wt.	^{35}S-rate	Dry wt.	^{35}S-rate
(F-a)	84.7*	1.9**	77.8*	1.9**	86.5*	1.7**
(F-b)	32.0	3.8	34.2	5.6	28.7	3.7
(F-c)	16.8	5.0	17.5	19.0	15.9	4.2
(F-d)	102.0	89.3	96.5	79.5	91.0	90.4

* Dry weight. mg/29 plants; ** % rate of total.

Discussions

Although numerous reports from various laboratories suggest that sulfur incorporation into plants is light dependent (5, 9, 10), the role of sulfur in the process of photosynthesis has not been thoroughly elucidated. Hanson et al. (11) reported that the rate of chloroplast protein-N/chloroplast protein-S remains approximately constant during the life cycle of sudan grass. There is no doubt that under certain conditions there is a direct relation between chlorophyll synthesis and sulfur metabolism.

Higher plants can not synthesize chlorophyll under dark condition, and their leaves become chlorotic in appearance. Etiolated leaves contain yellow plastids containing protochlorophyll. Studies by Smith (1) indicate that the chlorophyll is initiated from the protochlorophyll under light conditions. On the other hand, green plants become chlorotic by the lack of light. This is understood as the degradation of the chlorophyll during dark.

In our experiments, it is conceivable that the uptake mechanism of sulfate by a plant is not affected by treatment in light or dark. Up to three days duration, plants in their green state absorbed the same amount of sulfate in dark as in light. Abraham and Bachhawat (12) reported with *euglena* experiments, that light has no role so far as the sulfate uptake and activation is concerned. It will be true that the first step of the sulfur absorption has no direct connection with photosynthesis of plants. However, it is interesting that the exudation of absorbed sulfur from root to external solution is higher in the dark treatment of the green plant. The results remind us of the findings in previous experiments in which the higher sulfur exudation was recognized in normal plants (13). Nitrogen deficient plant revealed the least exudation of sulfur. It is difficult to elucidate the incompatible results in unified ideas. Even though the dark treatment of green plant results in higher soluble sulfur in the plant as in the nitrogen deficient plant, the situations of these two treatments would be in a different stage in plant metabolisms. On this concern, Nicolaef (14) reported on maize experiments that the maize plant root exuded higher quantities of sulfur, phosphorus and calcium to the external solution when the plant was transferred to a full nutrient solution. He concluded that the root exudation of sulfur as well as phosphorus by plants not only plays an important role in the sulfur nutrition of plants but the exudates directly effect absorption of nitrogen from a nutrient solution by plants.

The results presented show that the translocation rate of ^{35}S-sulfate was stimulated with light. Treatment of green plant in dark or etiolated plant in continuous darkness resulted in a relatively high ^{35}S-sulfate accumulation in the root and a low accumulation in the shoot. It can be emphasized that the general aspects of the absorption, translocation and assimilation of mineral elements confirmed the requirement of expenditure of energy in the above process (2–4).

The photosynthesis of plant will be the only energy soruce of these processes. In the previous section, the results evidently explained that the photosynthetic condition may affect the translocation of absorbed sulfate but not on the uptake process of sulfate by the plant.

Furthermore, the accumulation of absorbed ^{35}S-sulfur into the subcellular components of plant shows vigorous changes by the light or dark treatment. When the green normal plant was transferred into dark, the evident decrease of the protein fraction or chloroplast fraction was recognized following the increase of the inorganic soluble sulfur fraction. The soluble organic sulfur fraction was not so affected with this treatment. These changes of ^{35}S-sulfur accumulation were recognized particularly in the young leaves. When the green plant was transferred into dark, sulfur absorbed in older leaves was transported into the young upper leaves. From the data of the cellular sulfur analysis (Table 4), this transported sulfur into the young leaves originated mostly from the cytoplasmic non-protein sulfur of the old leaves. This may mean that the dark on green plant revealed young leaves in lower sulfur accumulation activity, but that in the fully developed old leaves, the accumulation of sulfur into the chloroplast or cytoplasmic soluble protein was not affected. A decrease of sulfur in non-protein fraction was recognized in this situation. In the experiment, a 72 hour dark treatment is not enough to give an evident effect on the chlorophyll or chloroplast synthesis or decomposition, but it is enough to give an effect on the balance of synthesis and decomposition. The accumulation of ^{35}S-sulfur into the cytoplasmic protein fraction was not relatively altered by the dark treatment. If the plants were kept in prolonged periods in dark, the distribution of sulfur in chloroplast protein would develop even in old leaves.

It is postulated by Smillie (15) that the light dependent growth of chloroplasts requires the synthesis of new ribosomal species and that this synthesis is initiated by light. On the contrary, Hase (16) reported that, in the etiolated chlorella cells, no ribosomal fraction was recognized. Therefore, the study of the cytoplasmic protein sulfur (ribosomes may be fractionated in this fraction under our procedures) will be the most important step to clarify the connection between sulfur metabolism and chlorophyll or chloroplast formation.

The beneficial effects of light on the etiolated leaves is due to the effect of light on the intake of minerals necessary for the formation of products, and not only the green leaves are affected by this, but also the etiolated leaves absorb nutrients by the participation of photosynthesis. The work of Kupke (17) indicates that the simultaneous appearance and parallel rise of protochlorophyll and cytoplasmic protein during development of bush bean seedlings lend circumstantial support to the evidence that there is a functional connection between these two components in chloroplast synthesis. The data in Tables 1 and 6, therefore, may be interpreted to mean that cytoplasmic protein is a building material of the chloroplast and

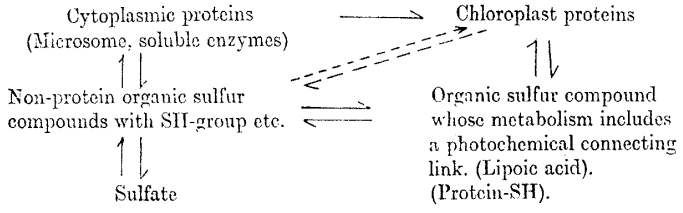
participates in the formation of chlorophyll under light conditions. It can be seen from the results that the dark treatment of the green excised leaves resulted in decreasing the incorporation of sulfur into the cytoplasmic protein fraction with decreasing of the dry weight of the fraction. On the contrary, when the etiolated leaves were transferred to the light condition, the evident increase of the incorporation of sulfur into the cytoplasmic protein fraction of leaves suggests that light has a direct relation to chloroplast protein formation, and presumably sulfur metabolism will connect to the energy metabolism of plant through the process of the cytoplasmic protein synthesis.

Recent knowledge of the mechanism of the chloroplast protein synthesis assumed that the isolated chloroplasts incorporate radioactive amino acids into their protein fraction (18), and that chloroplasts can synthesize their own protein moieties. There is, however, little evidence available to show whether the chloroplasts do, in fact, play an active part in the synthesis of proteins in the living cell. Regarding this, Racusen and Hobson (19) concluded from experiments with ^{14}C incorporation into plant leaves that, during photosynthesis with $^{14}CO_2$, the labeled bulk proteins of the chloroplasts and cytoplasm are quite similar with regard to both rate and kind of ^{14}C-amino acid incorporated. We may conclude from the experiments with regard to the conclusions of other workers (20), that the first important site of the sulfur metabolism in the plant was connected with the process of the chloroplast protein synthesis. The sulfur metabolism which is connected with the cytoplasmic protein synthesis may follow after the chloroplast protein synthesis.

Recently, Calvin (21) advanced a view that the primary effect of the absorption of a quantum of light in chlorophyll granules might cause the rupture of the disulfide bond in the lipoic acid molecule. At this time, lipoic acid metabolism has not yet been subjected to study, but if assuming that the formation and transformation of these disulfide compounds after incorporation into the photochemical connecting links will suggest the importance of the synthesis assignable to other sulfur compounds whose presence, in turn, may lead to the accumulation of protein sulfur, and also will suggest the necessity of light on the accumulation of sulfur.

Bandurski's group (22–24) reported another type of disulfide compound similar to the lipoic acid from yeast and discussed their SH-compound with regard to the sulfate reduction mechanism.

The above mentioned may be illustrated by the simplified schematic arrangement as follows:



The experimental data and literature examined in connection with the present research lead to the conclusion that the process of the reduction of sulfate in the plant proceeds not along one direction, but rather along multi-branched pathways. The process under discussion is accomplished not only at the expense of the oxidation of carbohydrate and other substances, but also at the expense of a more direct utilization of photochemical reactions by individual parts of plant tissues. In relation to the multiplicity of pathways available, the process of reduction or assimilation of sulfate, judged on the bases of data at hand in the literatures, displays an overall correspondence with the process of chloroplast protein synthesis, as well as a clear-cut specific difference.

Summary

Experiments on the effect of light or darkness on the utilization of sulfur in green or etiolated barley seedlings were conducted.

1. The transport of radio-sulfate into the shoot from the root was decreased by the lack of light supply. The higher assimilation of inorganic sulfate-^{35}S by means of the prolonged periods of darkness was observed.

2. The transported radio-sulfur in the etiolated barley shoot was assimilated more into the particulate fraction and cytoplasmic protein fraction under light conditions, but under continuous dark the etiolated barley shoot assimilated the radio-sulfur into soluble non-protein fraction.

3. Green barley seedlings which absorbed radio-sulfate were transferred to the non-radioactive normal nutrient solution and cultured further under light and dark conditions. With the lack of light supply, transport of radio-sulfur was dominant into the young leaves and, under continuous light condition, dominant in old leaves. Transported radio-sulfur into young leaves under dark condition was assimilated in cytoplasmic non-protein fraction. Young leaves under light condition assimilate the radio-sfulfur into cytoplasmic particulates and soluble protein fraction.

4. The leaves of etiolated and green barley seedlings were excised after the radio-sulfate was introduced through the roots and incubated. The etiolated leaves assimilated the radio-sulfur into cytoplasmic particulates and soluble protein fraction utilizing the soluble non-protein sulfur fraction during re-greening process while the etiolated leaves in dark condition did not. The green excised leaves with continuous supply of light assimilate radio-sulfur into cellular components from soluble non-protein sulfur.

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